*Journal of Physiology* (1991), **434**, *pp.* 423–440
*With 6 figures*
*Printed in Great Britain*



# SUBSTRATES FOR MUSCLE GLYCOGEN SYNTHESIS IN RECOVERY FROM INTENSE EXERCISE IN MAN

BY J. BANGSBO, P. D. GOLLNICK, T. E. GRAHAM AND B. SALTIN

*From the August Krogh Institute, Universitetsparken 13, DK-2100 Copenhagen Ø, Denmark*

(*Received 2 July 1990*)

## SUMMARY

1. Intramuscular glyconeogenesis from lactate after intense exercise was examined by using the one-legged knee extension model which enables evaluation of metabolism in a well-defined muscle group.

2. In seven subjects measurements of leg blood flow and arterial–venous differences of various substrates were performed in individuals after intense, exhaustive knee extensor exercise lasting 3·0 min. Muscle glycogen and lactate concentrations were determined in the quadriceps muscle immediately after exercise and three times during 1 h of recovery.

3. Muscle glycogen increased from 93·7 ± 6·7 (± S.E.M.) to 108·8 ± 8·1 mmol (kg wet wt)$^{-1}$ during the recovery period. Muscle lactate was 27·1 ± 2·1 mmol (kg wet wt)$^{-1}$ at the end of exercise and decreased to 14·5 ± 2·1, 6·7 ± 1·1, and 3·0 ± 0·5 mmol (kg wet wt)$^{-1}$ after 3, 10 and 60 min of recovery, respectively.

4. More than two-thirds of the lactate that accumulated in the muscle during the intense exercise was released into the blood. It was estimated that between 13 and 27% of the lactate could have been converted to glycogen. This corresponded to a glycogen resynthesis rate from lactate of 0·17–0·34 and 0·002 mmol glucosyl units min$^{-1}$ (kg wet wt)$^{-1}$ for the first 10 and last 50 min of recovery, respectively.

5. The $O_2$ debt of the leg was 1·5 l of which the resynthesis of ATP, creatine phosphate (CP) and glycogen and reloading of haemoglobin (Hb) and myoglobin (Mb) only could account for one-third. It is proposed that the elevated oxygen uptake during recovery is linked to the metabolic use of intramuscular triacylglycerol.

## INTRODUCTION

The fate of the lactate that has accumulated in active skeletal muscle at the termination of intense, exhaustive exercise has not been resolved. It can leave the muscle, as well as being metabolized within the muscle, with one possibility being that it is a substrate for intramuscular glyconeogenesis (for references see Brooks, 1985), which contributes to the oxygen repayment (oxygen debt) in the recovery from the intense exercise.

In the 1920s Meyerhof and co-workers (1920) studied frog muscles and found that about 75% of the lactate that accumulated in a muscle during electrically induced

contractions was converted to glycogen within the muscle during recovery. Some 40 years later Krebs challenged this finding on the basis that the activities of the key enzymes needed for gluconeogenesis with lactate as substrate were too low in skeletal muscle to allow for a significant lactate removal through this pathway (Krebs & Woodford, 1965). However, Opie & Newsholme (1967) discovered sufficient quantities of the necessary enzymes in frog muscle. Later Bendall & Taylor (1970) demonstrated that glycogen can be formed in large amounts from lactate, added to isolated frog sartorius muscles. It seems also to occur in human skeletal muscle. Thus, Hermansen & Vaage (1977) estimated that 90% of the lactate in the muscle after intense exercise remained within it and at least 75% of the lactate was converted to glycogen. Further support for this notion was obtained by Åstrand and co-workers (Åstrand, Hultman, Juhlin-Danfelt & Reynolds, 1986), who concluded that about 50% of the lactate produced during exercise was used to synthesize glycogen. Thus, estimates of post-exercise lactate metabolism suggest that human skeletal muscle possesses the necessary biochemical machinery for synthesis of glycogen directly from lactate. Further support for this concept comes from studies using rodent muscle. McLane & Holloszy (1979) found that [$^{14}$C]lactate was incorporated into glycogen in the isolated perfused rat hindlimb, and recently Bonen, McDermott & Tan (1990) reported that *in vitro* mouse muscle derived from lactate 5–32% of the glycogen synthesized during recovery.

Although these studies have limitations in their methodology they demonstrate that glycogen synthesis from lactate appears to occur within the muscle. However, it is not clear whether this metabolic pathway is the dominant one in man. Thus, we have re-investigated the hypothesis that glyconeogenesis from lactate makes a significant contribution to the restoration of muscle glycogen after high-intensity exercise.

## METHODS

### *Subjects*

Seven healthy male subjects ranging in age from 22 to 27 years, with an average height of 180 cm and an average weight of 71 kg, participated in the experiment. Five of the subjects had participated in previous experiments of similar design and measurements. All subjects were habitually physically active, but none trained for competition. The subjects were fully informed of any risks and discomfort associated with these experiments before giving their informed consent to participate. The study was approved by the local ethical committee.

### *Methods*

*Blood flow.* Femoral venous blood flow was measured by the thermodilution technique (Andersen & Saltin, 1985). Briefly, ice-cold saline was infused at a constant rate into the femoral vein for 10–15 s. At rest and in late recovery, when the blood flow was low, a 30–45 s infusion period was used (Richter, Mikines, Galbo & Kiens, 1989). Bolus injections of ice-cold saline were also used in these low-flow situations (Gaffney, Sjøgaard & Saltin, 1990).

*Blood analysis.* Oxygen saturation of blood was determined spectrophotometrically (Radiometer OSM-2 Hemoximeter). Haemoglobin concentration was determined with the Hemoximeter which was calibrated spectrophotometrically by the cyanomethaemoglobin method (Drabkin & Austin, 1935). Haemoglobin (Hb) concentrations at low oxygen saturation were adjusted with a correction factor (measured Hb − (100 − measured Hb saturation (%)) × 0·01) obtained from multiple measurements of oxygen content of fully oxygenated blood samples as determined by Van Slyke analysis (Holmgren & Pernow, 1959). $P_{O_2}$, $P_{CO_2}$ and pH were measured with the Astrup technique (ABL 30, Radiometer, Copenhagen, Denmark). Lactate and glucose were analysed from PCA-

precipitated extractions of the blood samples using a fluorometric assay (Lowry & Passonneau, 1972). Free fatty acids (FFA) and glycerol concentrations in plasma were determined according to the principles of Shimizu, Inoue, Tani & Yamada (1979). Plasma amino acids, derivatized with phenylisothiocyanate, were determined in duplicate by high-performance liquid chromatography (HPLC) (Heinrikson & Meredith, 1984).

*Whole-body metabolism.* Pulmonary oxygen uptake ($\dot{V}_{O_2}$) and carbon dioxide release ($\dot{V}_{CO_2}$) were determined by collection of expired air in Douglas bags. The volume of air was measured in a Tissot spirometer and the concentrations of $O_2$ and $CO_2$ were determined with Servomex and Beckman LB-2 analyser, respectively. The instruments were regularly calibrated (Scholander technique) with known gas mixtures. The respiratory expiratory ratio (RER) was calculated as the ratio between the pulmonary $\dot{V}_{CO_2}$ and $\dot{V}_{O_2}$.

*Muscle mass.* Surface measurements of the subjects thigh length ($L$) and circumferences ($O_1$, $O_2$, and $O_3$) were performed together with skinfold ($S$) measurements of the thigh. Thigh volume ($V$) was then calculated from the formula:

$$V = L \times (12\pi)^{-1} \times [(O_1)^2 + (O_2)^2 + (O_3)^2] - (S - 0{\cdot}4) \times 2^{-1} \times L \times (O_1 + O_2 + O_3) \times 3^{-1},$$

(Jones & Pearson, 1969). The quadriceps femoris muscle mass ($M$) was then calculated as:

$$M = 0{\cdot}307 \times V + 0{\cdot}353 \; (n = 12, \; r = 0{\cdot}93, \; P < 0{\cdot}001),$$

(autopsy study, O. Halskov, personal communication).

This antropometric approach gave values similar to those from estimations based on multiple CAT-Scans (Saltin, 1985). The present subjects had a mean knee extensor mass of 3·0 kg, with a range of 2·6–3·5 kg.

*Muscle biopsies*

Muscle samples were analysed for total water by weighing the samples before and after freeze drying and for lactate, glycogen and creatine phosphate (CP) by fluorometric assays (Lowry & Passonneau, 1972). ATP and IMP (inosine monophosphate) concentrations were determined with a HPLC technique (Magnson & Perryman, 1980). The remainder of the muscle was analysed enzymatically for glucose, glucose-1-phosphate (G-1-P), glucose-6-phosphate (G-6-P), fructose-6-phosphate (F-6-P), and alanine as described by Harris, Hultman & Nordesjö (1974).

Changes in muscle variables were calculated on dry weight basis, and normalized to the water content of resting muscle as the muscle mass was determined at rest. This makes the muscle values comparable to the exchanges of substrates between femoral blood and the quadriceps muscles.

*Procedures*

Subjects performed one-legged exercise in the supine position on an ergometer that permitted the exercise to be confined to the quadriceps muscles (Andersen, Adams, Sjøgaard, Thorboe & Saltin, 1985). At the time of the experiment a catheter was placed in the femoral artery with the Seldinger technique with the tip placed 1–2 cm proximal to the inguinal ligament. The tip of one of the two femoral vein catheters was positioned approximately 8 cm in the retrograde direction, i.e. 12–14 cm distal to the inguinal ligament. This catheter was used for collecting blood samples and for infusing the ice-cold saline. Another venous catheter was placed in the inguinal region with the tip 1–2 cm distal to the ligament. The thermistor for measurement of venous blood temperature was inserted through this catheter and was advanced just proximal to the tip. During the exercise and recovery period a cuff just below the knee was inflated to occlude blood flow to and from the lower leg. The cuff was released for very brief periods ($< 30$ s) in the recovery at times not likely to interfere with the measurements (see below).

*Protocol*

On the morning of the experiment, subjects arrived after a light breakfast. Placement of catheters was followed by 30 min of rest in the supine position.

Then warm up exercise was performed with the experimental leg for 10 min at an intensity corresponding to about 25% of peak aerobic effect of the leg (10 W). After at least 10 min rest a muscle biopsy was taken from m. vastus lateralis and blood was drawn simultaneously from the femoral artery and vein. Next an intense work load (mean, 65; range, 52–79 W) was performed to exhaustion (mean, 3·0 min; range, 2·0–4·9 min) followed by a recovery period of 1 h.

At the end of the intense exercise blood flow was measured and blood samples from the femoral artery and vein were taken. During early recovery (0–5 min), blood flows were determined as frequently as possible, followed by the collection of blood samples. At least two complete measurements per 90 s were accomplished. Beyond the 5 min of recovery, blood flows were measured and blood samples were collected at about 7, 10, 13, 16, 20, 30, 45 and 60 min of recovery. Lower leg circulatory occlusion was maintained during the recovery period with the exception of four to five 'breaks' placed at about 0·5, 8, 25, 40 and 50 min. Expired air was continuously collected in Douglas bags during the exercise and recovery period. Additional muscle biopsies were taken at exhaustion and after 3, 10 and 60 min of recovery.

*Calculations*

*Determination of blood $CO_2$ content.* Arterial and venous whole blood content was determined from blood Hb, temperature, saturation, pH, and $P_{CO_2}$ according to the calculation described by Douglas, Jones & Reed (1988).

*Leg $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$ and substrate exchange.* $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$, net lactate, net glucose, net glycerol, net FFA, and net amino acid exchange by the thigh were calculated by multiplying the blood flow, or for the last three variables plasma flow, by the difference between femoral artery and venous ($a-v_{fem}$) concentrations of the variables. A continuous blood flow curve was constructed for each subject by linear connection of the consecutive data points to obtain time-matched values for the blood flow measurements with the latter two variables. No difference between 'time-matched' and measured blood flow was larger than 0·3 l min$^{-1}$ during early recovery, while the difference was less than 0·1 l min$^{-1}$ in the last stage of recovery. The leg respiratory quotient (RQ) was calculated as the ratio between the leg $\dot{V}_{CO_2}$ and $\dot{V}_{O_2}$.

*Total $V_{O_2}$, total $V_{CO_2}$ and total exchange of substrates.* The total $V_{O_2}$, $V_{CO_2}$, net lactate, net glucose, net glycerol, net FFA, and net amino acid exchange during the first 3 min, the following 7 min or the last 50 min of recovery are given by the time integral

$$\int_x^y \mathrm{f}(t)\,\mathrm{d}t,$$

where $x$ is start of recovery (0 min), 3 min or 10 min, $y$ is either 3 min, 10 min or end of recovery (60 min), and f($t$) is exchange of one of the variables at a given time ($t$) during recovery. In practice, the exchanges were determined as the areas under f($t$) curves, with time on the $x$-axis. The curves are produced on the assumption that there was a linear relationship between two measured values. When the above approach for calculations was compared with the method of matching blood flow with blood concentration measurements nearest in time, the largest difference in net exchange of any variable within the recovery periods was 4 %. In most instances deviations were less than 1 %.

*Substrate fluxes by the hamstring/adductor muscles*

One problem which exists with the calculation of substrate fluxes for the quadriceps muscles is that a fraction of the blood flow measured in the femoral vein has perfused the hamstring and adductor muscles. An uptake of a substrate by these muscles lowers the femoral venous concentration and subsequently results in the underestimation of the release of that substrate from the quadriceps muscles. During exercise of the quadriceps muscles and early recovery the blood flow is undoubtedly different in these muscle groups, with the hamstring/adductors blood flow being very small compared to that of the quadriceps. However, the longer the recovery period the less discrepancy there will be between the flows to the muscle groups.

In order to examine how critical the metabolism of the resting hamstring/adductor muscles is for the present calculations an additional study was performed. Two subjects exercised with the quadriceps muscles of one leg to generate changes in arterial concentrations of relevant metabolites. At rest, and both during and the first 10 min after the exercise $a-v_{fem}$ differences for lactate, glucose, glycerol, FFA, amino acids, and oxygen were determined for the contralateral resting thigh. These data were used to estimate the net substrate exchange of the hamstring/adductor muscles after exercising with the ipsilateral quadriceps muscles. It was assumed that the metabolism of the hamstring/adductor muscles responded in a fashion similar to the contralateral resting thigh.

Based on Wesche's (1986) findings that flow at rest is directed with $\frac{1}{3}$ to skin and tissues other than muscle and with an equal share to extensors and flexors/adductors, the estimated total net uptake of lactate in the latter muscles amounted to 5·1 mmol with the uptake being 1·3, 2·4 and 1·4 mmol during the first 3 min, next 7 min and last 50 min of recovery, respectively (Fig. 1). Total

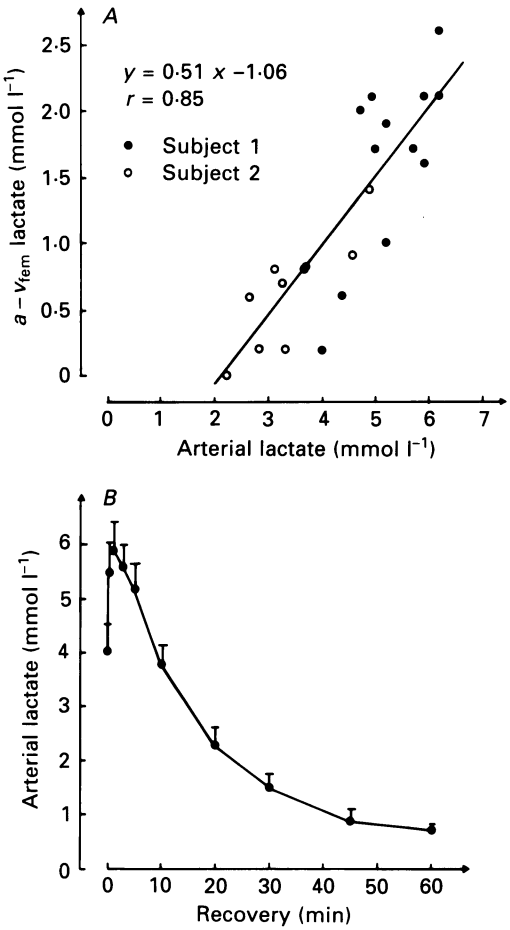


Fig. 1. Arterial lactate concentration during the 1 h of recovery (*B*) and the relationship between arterial concentrations and $a-v_{fem}$ lactate difference for a resting thigh before, during and after exercising with the contralateral quadriceps muscles (*A*). Based on these measurements and an assumed equal blood flow to the hamstring/adductor muscles and quadriceps muscles (see Methods) the uptake of lactate by the hamstring/adductor muscles at each time point of recovery was calculated, and subsequently the total uptake of lactate during three time periods of recovery was determined by using the calculation described in Methods.

net glucose uptake during the 1 h of recovery was 2·7 mmol, while the net uptake of FFA, glycerol and amino acids were insignificant (< 0·01 mmol).

*Statistics*

Differences between pre-exercise and recovery values were determined by the Wilcoxon ranking test for paired data (Pratt's modification; Siegel, 1965).

## RESULTS

### *Muscle glycogen and lactate*

The muscle glycogen concentration immediately after exercise was 93·7 ±6·7 mmol (kg wet wt)$^{-1}$ (mean ± S.E.M.) and after 1 h of recovery it had increased to 108·8 ± 8·1 mmol (kg wet wt)$^{-1}$, which was 90% of the pre-exercise value ($P > 0·05$). In total, glycogen synthesis for the quadriceps muscle was 43·5 ± 13·9 mmol. The water content of the muscle was 75·3 ± 0·2% before the exhaustive exercise and it increased to 77·4 ± 0·3% at exhaustion and there was only a small reduction during the recovery period.

Muscle lactate concentration immediately after exercise was 27·1 ± 2·1 mmol (kg wet wt)$^{-1}$. This declined to 14·5 ± 2·1 mmol (kg wet wt)$^{-1}$ during the first 3 min of recovery. During the remaining period of recovery lactate disappeared at a lower rate, the muscle lactate concentrations were 6·7 ± 1·1 and 3·0 ± 0·5 mmol (kg wet wt)$^{-1}$ after 10 and 60 min of recovery, respectively. Total reduction in muscle lactate was 24·1 mmol (kg wet wt)$^{-1}$, which, when adjusted for the change in water content of the muscle tissue, was equivalent to 26·2 mmol (kg wet wt)$^{-1}$ (Fig. 2).

### *Leg blood flow*

Leg blood flow was 4·3 l min$^{-1}$ at the end of exercise, and decreased slowly during recovery to 3·1, 1·9 and 1·2 l min$^{-1}$ after 1, 5 and 10 min, respectively. At 30 min and during the rest of the 1 h of recovery it was similar ($P > 0·05$) to the pre-exercise value of 0·5 l min$^{-1}$.

### *Lactate efflux*

During the early phase of recovery the lactate in arterial and femoral venous blood continued to increase, reaching 5·9 and 8·0 mmol l$^{-1}$, respectively, at 1 min post-exercise. The rise was greater in the femoral arterial blood, resulting in the $a-v_{fem}$ lactate difference declining from 4·4 mmol l$^{-1}$ at exhaustion to 2·1 mmol l$^{-1}$ at 1 min of recovery. Further reductions occurred during the remaining recovery period with values of 1·9, 0·6 and 0·2 mmol l$^{-1}$ observed at 3, 10 and 60 min, respectively.

Net leg lactate efflux was 15·7 mmol min$^{-1}$ at exhaustion, decreased rapidly to 8·3 and 2·5 mmol min$^{-1}$ after 1 and 5 min, respectively. However, it was still above 0·5 mmol min$^{-1}$ after 10 min and was slightly higher ($P < 0·05$) than the pre-exercise value after 20 min and remained so during the rest of the recovery period (Fig. 3). The total net lactate release of the first 3 min, next 7 min, and last 50 min of recovery was 25·8, 16·8 and 9·6 mmol, respectively (Table 1). This lactate release accounted for 64% of the accumulated muscle lactate at the end of exercise. To this total release should be added the 5·1 mmol lactate uptake by the hamstring/adductor muscles (see Methods), which means that 70% can be accounted for by release of lactate into the blood.

Hence, less than one-third, or 21·3 mmol, of the muscle lactate which disappeared was metabolized within the muscle. Of this, 51% was metabolized within the first 3 min of recovery with a mean rate of 1·20 mmol min$^{-1}$ (kg wet wt)$^{-1}$, 46% during the next 7 min with a mean rate of 0·47 mmol min$^{-1}$ (kg wet wt)$^{-1}$, and 3% in the last 50 min period with a mean rate of 0·004 mmol min$^{-1}$ (kg wet wt)$^{-1}$.

*Exchange of other substrates*

Glucose uptake by the exercising leg was 0·50 mmol $min^{-1}$ at the end of exercise and it increased slightly during early recovery being 0·92 mmol $min^{-1}$ 3 min into the recovery. Thereafter it decreased to 0·17 mmol $min^{-1}$ at 10 min and was nearly

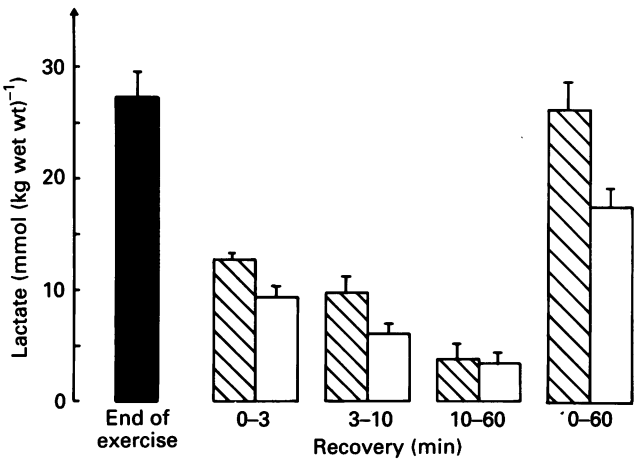


Fig. 2. Muscle lactate concentration immediately after intense exercise (■), net decrease in muscle lactate (▨) and net lactate release to the blood (□) of one thigh during 1 h of recovery (0–60 min) divided into three time periods (0–3, 3–10, and 10–60 min). The mass of the knee extensor muscles amounted to 3·0 kg on average.

constant for the remainder of the recovery period (Fig. 3). The total net leg glucose uptake by the thigh was 20·9 mmol during the 1 h of recovery (Table 1), from which the uptake by the hamstring/adductor muscles should be deducted (see Methods). Thus, the net uptake of the quadriceps muscles was 18·2 mmol.

A total net FFA uptake of 0·35 mmol was found, with a net release of 0·16 mmol during the first 10 min, and a net uptake of 0·51 mmol in the remaining 50 min of recovery (Fig. 4, Table 1).

The glycerol release was 0·011 mmol $l^{-1}$ at the end of exercise. It decreased transiently immediately after exercise to about nil, but then increased rapidly, and it remained high (0·055 mmol $l^{-1}$) for several minutes. Thereafter it decreased again and reached the pre-exercise value ($P > 0·05$) after approximately 45 min (Fig. 4). During the first 10 min of recovery 0·36 mmol of glycerol was released, and during the last 50 min it was 0·49 mmol (Table 1).

The amino acid responses during recovery were generally small and variable; the net leg release of amino acids during the hour was 1·74 ± 0·87 mmol, with glutamine (0·76 ± 0·38 mmol) and alanine (0·78 ± 0·55 mmol) being the dominant amino acids released. Alanine release for the first 3 and the following 7 min was 0·19 ± 0·10 and 0·21 ± 0·20 mmol, respectively. The branched chain amino acids were taken up in small quantities (0·31 ± 0·10 mmol).

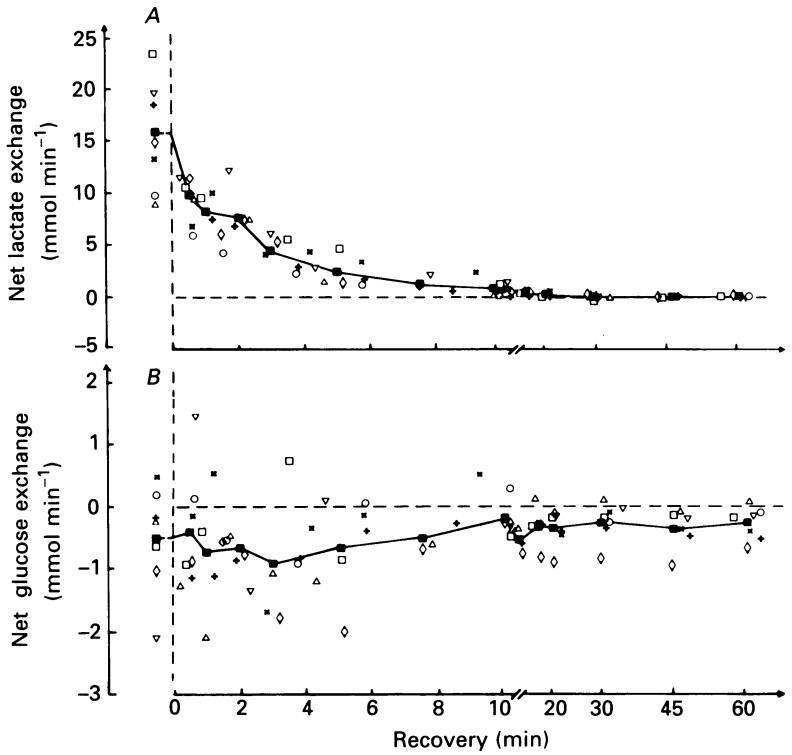


Fig. 3. Individual and mean values for leg lactate (*A*) and glucose exchange (*B*) during 1 h of recovery. Positive values mark release and negative values uptake. The various symbols represent one individual except the filled circles which give the mean values. The horizontal and vertical lines represent no net exchange and the onset of recovery, respectively.

TABLE 1. Net uptake* and release* of various substrates of one thigh during 1 h of recovery after intense exercise to exhaustion

| | Recovery (min) | | | |
|---|---|---|---|---|
| | 0–3 | 3–10 | 10–60 | 0–60 |
| Glucose uptake (mmol) | 2·0 ± 0·5 | 3·2 ± 1·3 | 15·7 ± 5·1 | 20·9 ± 5·9 |
| Lactate release (mmol) | 25·8 ± 2·3 | 16·8 ± 1·9 | 9·6 ± 2·0 | 52·2 ± 4·6 |
| Glycerol release (mmol) | 0·06 ± 0·01 | 0·30 ± 0·05 | 0·49 ± 0·05 | 0·85 ± 0·07 |
| FFA uptake (mmol) | −0·05 ± 0·15 | −0·11 ± 0·27 | 0·51 ± 0·21 | 0·35 ± 0·27 |

Values are means ± S.E.M.

* The uptake or release of the hamstring/adductor muscles is not included in these values (see Methods).

*Leg oxidation*

Leg $\dot{V}_{O_2}$ at the end of exercise was 611 ml min$^{-1}$ and fell quickly in the start of recovery to 221, 109 and 30 ml min$^{-1}$ after 0·5, 1 and 10 min, respectively. In the remaining part of recovery the decline in leg $\dot{V}_{O_2}$ was slow, not reaching ($P > 0·05$) the

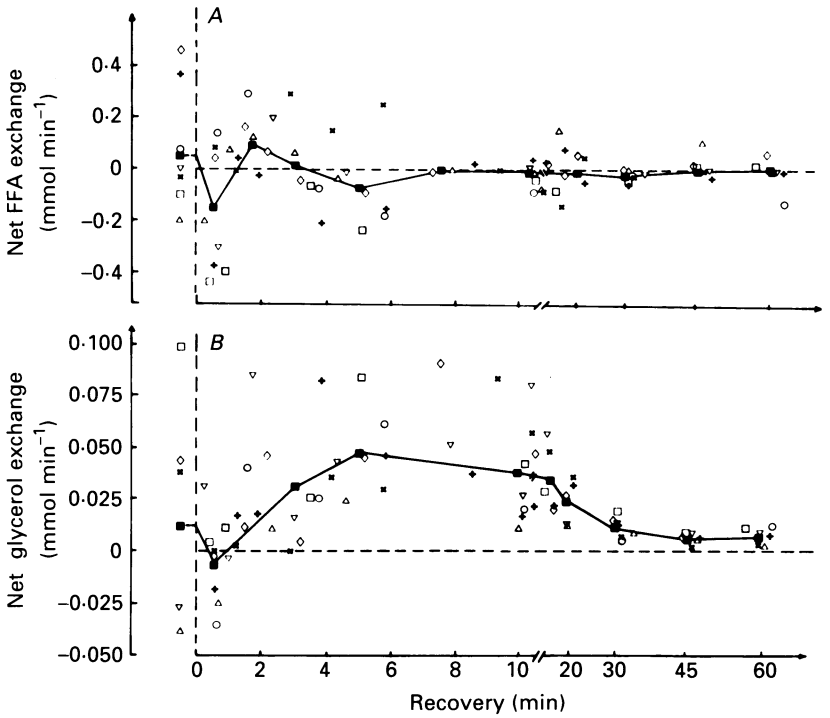


Fig. 4. Individual and mean values for leg FFA (*A*) and glycerol exchange (*B*) during 1 h of recovery. Positive values mark release and negative values uptake. The various symbols represent one individual except the filled circles which give the mean values. The horizontal and vertical lines represent no net exchange and the onset of recovery, respectively.

TABLE 2. Individual leg and pulmonary $V_{O_2}$, $V_{CO_2}$ and RQ (RER) during intense exercise and the following 1 h of recovery

| | Leg | | | | | Pulmonary | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Exercise | | Recovery | | | Exercise | | Recovery | | |
| Subject | $V_{O_2}$ (l) | $V_{CO_2}$ (l) | $V_{O_2}$ (l) | $V_{CO_2}$ (l) | RQ* | $V_{O_2}$ (l) | $V_{CO_2}$ (l) | $V_{O_2}$ (l) | $V_{CO_2}$ (l) | RER* |
| 1 | 2·79 | 2·75 | 1·77 | 1·46 | 0·80 | 10·21 | 8·41 | 18·81 | 13·54 | 0·75 |
| 2 | 1·07 | 1·26 | 2·00 | 2·06 | 1·13 | 3·70 | 3·82 | 20·41 | 14·76 | 0·73 |
| 3 | 1·23 | 1·26 | 2·68 | 1·90 | 0·72 | 3·11 | 4·33 | 24·47 | 17·43 | 0·76 |
| 4 | 1·07 | 1·24 | 3·35 | 2·70 | 0·86 | 3·16 | 2·90 | 24·68 | 20·35 | 0·81 |
| 5 | 0·75 | 1·10 | 3·03 | 1·94 | 0·76 | 2·20 | 2·94 | 18·13 | 12·84 | 0·75 |
| Mean | 1·46 | 1·52 | 2·71 | 2·00 | 0·78 | 3·99 | 4·48 | 21·30 | 15·78 | 0·76 |
| S.E.M. | 0·45 | 0·31 | 0·34 | 0·26 | 0·03 | 0·98 | 1·02 | 1·39 | 1·38 | 0·01 |
| *n*† | 4 | 4 | 4 | 4 | 4 | 5 | 5 | 5 | 5 | 5 |

* The $CO_2$ production in the calculation of mean leg RQ and mean pulmonary RER during recovery are $V_{CO_2}$ during recovery plus extra $CO_2$ release during exercise (calculated from $V_{O_2}$ and $V_{CO_2}$ during exercise under the assumption that RQ = 1 during exercise) as it is assumed that the $CO_2$ stores are replenished during recovery.

† As the calculated leg RQ for subject no. 2 was unphysiological due to two unrealistic high $P_{CO_2}$ measurements, his values were not included in the calculation of mean.

pre-exercise value of 16 ml $min^{-1}$ until 60 min. The $V_{O_2}$ by the leg during the first 3, next 7 and last 50 min of recovery was 0·47, 0·33 and 1·71 l, respectively, or 12·1 ± 1·4 % of the whole body $V_{O_2}$ of 20·7 l in the recovery period. The corresponding leg $O_2$ debt ($V_{O_2}$ − pre-exercise $V_{O_2}$) was 0·42, 0·21 and 0·89 l for the three time periods, respectively, and the pulmonary $O_2$ debt was 4·5 l for the entire 60 min.

TABLE 3. Knee extensor (weight 3·0 kg) substrate utilization during 1 h of recovery after intense exercise to exhaustion

| | |
|---|---|
| A Carbohydrate metabolism (glucosyl units) | |
| Use | |
| Muscle glycogen resynthesis (mmol) | 43·5 |
| CHO contribution to aerobic metabolism (mmol) | 5·5 |
| Total | 49·0 |
| Available | |
| Glucose uptake (mmol) | 18·2 |
| Lactate consumption (mmol) | 9·5 |
| Changes in glycolytic intermediates measured and muscle glucose (mmol) | 8·7 |
| Changes in glycolytic* intermediates not measured (mmol) | 9·0 |
| Total | 45·4 |
| B Fat-metabolism (mmol fatty acid) | |
| Use | |
| Lipid contribution to aerobic metabolism† (mmol) | 3·2 |
| Available | |
| Blood borne/intramuscular TG (glycerol release) (mmol) | 2·6 |
| Free fatty acid uptake (mmol) | 0·4 |
| Total | 3·0 |

* Data from Jones, McCartney, Graham, Spriet, Kowalchuk, Heigenhauser & Sutton (1985), Cheetham, Boobis, Brooks & Williams (1986), and McCartney, Spriet, Heigenhauser, Kowalchuk, Sutton & Jones (1986).

† 1 mmol palmitate ≈ 129 mmol ATP.

Leg $\dot{V}_{CO_2}$ was 885 ml $min^{-1}$ at the end of exercise, and decreased rapidly during recovery to 541, 218 and 17 ml $min^{-1}$ after ½, 1 and 10 min, respectively. In the remaining part of recovery it was below 15 ml $min^{-1}$. The mean $V_{CO_2}$ ($n = 5$) from the muscle during the first 3 min was 0·81 l, during the next 7 min 0·23 l, and during the remaining period of recovery it was 0·98 l.

Leg RQ was 2·00, 0·56, 0·64 and 0·69 after 1, 10, 20 and 60 min of recovery, respectively, and mean leg RQ was 0·78 ($n = 4$) for the 1 h of recovery (Table 2). Mean RER was 0·76, with averages of 1·04 (1·15–0·91) and 0·71 (0·69–0·76) for the first 10 and last 50 min, respectively. Based on leg RQ and $V_{O_2}$ the substrate contribution to oxidation was estimated to equal 5·5 mmol glucosyl units and 3·2 mmol FA for the 1 h of recovery (Table 3).

*Muscle metabolites*

Muscle glucose of 1·76 mmol (kg wet wt)$^{-1}$ at the end of exercise decreased to 0·33 mmol (kg wet wt)$^{-1}$ after 1 h of recovery. Also the G-1-P, G-6-P and F-6-P concentration which at the start of recovery averaged 0·04, 1·42 and 0·19 mmol

(kg wet wt)$^{-1}$, respectively, were lower at the end of the recovery period (0·01, 0·15 and 0·03 mmol (kg wet wt)$^{-1}$, respectively). The muscle alanine concentration decreased from 1·81 to 1·43 mmol (kg wet wt)$^{-1}$ during recovery.

The ATP concentration of the muscle immediately after exercise was $4{\cdot}2 \pm 0{\cdot}5$ mmol (kg wet wt)$^{-1}$, and increased to $4{\cdot}9 \pm 0{\cdot}5$, $5{\cdot}5 \pm 0{\cdot}2$ and $5{\cdot}7 \pm 0{\cdot}4$ mmol (kg wet wt)$^{-1}$ (90% of the pre-exercise value; $P > 0{\cdot}05$) after 3, 10 and 60 min of recovery, respectively. The CP concentration which averaged $7{\cdot}6 \pm 0{\cdot}5$ mmol (kg wet wt)$^{-1}$ at the start of recovery, was elevated to $12{\cdot}5 \pm 2{\cdot}1$ mmol (kg wet wt)$^{-1}$ at 3 min of recovery. In the remaining recovery period the CP concentration increased slowly to $17{\cdot}1 \pm 2{\cdot}0$ mmol (kg wet wt)$^{-1}$ after 10 min and to $19{\cdot}6 \pm 1{\cdot}0$ mmol (kg wet wt)$^{-1}$ after 60 min.

## DISCUSSION

The major finding of this study is that more than two-thirds of the lactate that accumulated in the muscle during the intense exercise was released to the blood during the recovery. This assumes that lactate production ceased when exercise stopped. Thus, approximately 21 mmol of the accumulated lactate at exhaustion was available for metabolism within the muscle. The question is how much of this was converted to glycogen?

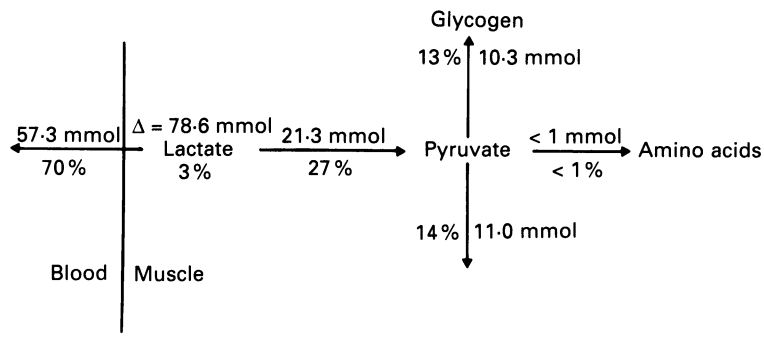


Fig. 5. Fate of intramuscular lactate accumulated at the end of exhaustive exercise in quadriceps muscles. For details see start of Discussion. The vertical line on the left of the figure signifies the blood–muscle interface.

The lactate removed within the muscle could (via pyruvate) have followed several routes (Fig. 5). One is conversion to alanine. Alanine concentration in the muscle was reduced by 0·4 mmol (kg wet wt)$^{-1}$ during recovery, and during that time the alanine release was 0·25 mmol (kg wet wt)$^{-1}$. The exchange of amino acids whether release or uptake was less than 0·1 mmol min$^{-1}$, and the total net exchange was less than 0·6 mmol (kg wet wt)$^{-1}$. Though these data are not a precise determination of lactate removal by amino acid production, they suggest that this pathway was quantitatively quite small, which is in agreement with Felig & Wahren (1974).

To be able to give estimates for the extent to which pyruvate produced from lactate is either oxidized or used for glycogen synthesis the whole metabolic situation

has to be considered. Carbohydrate (pyruvate) contributed 5·5 mmol glucosyl units to oxidation during the 1 h of recovery, and the majority appears to have been oxidized during the first 10 min, as during the remaining 50 min the leg RQ was below 0·70 and RER was 0·71. The origin of this pyruvate could be the muscle lactate and if so 14% of the lactate accumulated at the end of exercise was metabolized via this route and 13% or 10·3 mmol was used to glyconeogenesis (Fig. 5). Glycolytic intermediates accumulated within the muscle or glucose taken up from the blood stream could also have contributed to the pool of carbohydrate oxidized. If they account for the entire carbohydrate oxidation which took place in the recovery, 27% of the lactate present in the muscle at exhaustion was converted to glycogen. Our data do not allow us to determine what portion of the oxidized pyruvate is derived from lactate. However, the oxidation occurs in the first 10 min when there is the greatest potential to form pyruvate from lactate due to the high concentration of the latter, which suggests that lactate is the major source of the pyruvate oxidized in the mitochondria. This notion is supported by the findings of Bendall & Taylor (1970) that the glycogen synthesis equalled the lactate available for gluconeogenesis, when the latter was calculated based on the assumption that only lactate was the substrate for carbohydrate oxidation. Thus, the contribution of lactate to gluconeogenesis was probably closer to the 13% than the 27% of the lactate accumulated at the end of exercise.

Taken together, the available lactate, the glycolytic intermediates, the glucose in the cell and the blood glucose taken up amount to 45·4 mmol glucosyl units, which after subtraction of what is oxidized can account for 39·9 mmol glucosyl units of the observed glycogen storage of 43·5 mmol or 92% (Table 3A). Thus, glycolytic intermediates and especially blood glucose played major roles as substrates for the resynthesis of glycogen. These results are in agreement with findings for the rat hindlimb (Shiota, Golden & Katz, 1984), where it was demonstrated that glucose was the dominant substrate for glycogen synthesis in recovery after electrical stimulation of the leg muscles, and with Bonen *et al.* (1990), who observed that incubated mouse muscles primarily formed glycogen from glucose with only 5–32% of the glycogen being derived from lactate.

The finding that between 13 and 27% of the lactate present in the muscle at the end of exercise could have supported glycogen synthesis is considerably less than the estimates of 50 and 75% presented by Åstrand *et al.* (1986) and Hermansen & Vaage (1977), respectively. The average rate of glycogen synthesis of 0·25 mmol glucosyl units $min^{-1}$ (kg muscle)$^{-1}$ for the 60 min of recovery in the present study was similar to the rates of 0·32 and 0·51 mmol glucosyl units $min^{-1}$ $kg^{-1}$ (for 30 min) reported by Åstrand *et al.* (1986) and Hermansen & Vaage (1977), respectively. Furthermore, the muscle lactate disappearance rate of 2·24 mmol $min^{-1}$ $kg^{-1}$ for the first 10 min and 0·08 mmol $min^{-1}$ $kg^{-1}$ for the last 50 min of recovery (mean, 0·40 mmol $min^{-1}$ $kg^{-1}$) in the present study was of similar magnitude to that reported by Åstrand *et al.* (1986) (0·36 mmol $min^{-1}$ (kg wet wt)$^{-1}$ and by Hermansen & Vaage (1977) (0·74 mmol $min^{-1}$ (kg wet wt)$^{-1}$ for a 30 min period).

One major difference between the present study and that of Åstrand *et al.* (1986) is the method for quantifying the lactate efflux from the muscle. They estimated the muscle lactate release based on the whole-body clearance of lactate from the

circulation. In contrast, in the present study a direct Fick calculation of lactate release was employed. Similarly there are methodological differences for the key measurements between the present study and that of Hermansen & Vaage (1977). They found, compared to the present study, a much lower lactate release from and

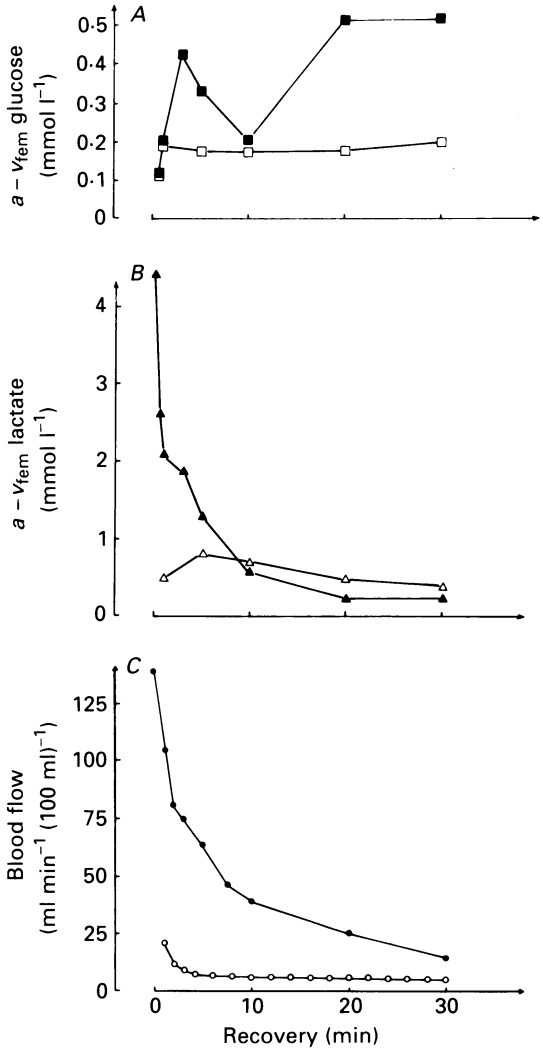


Fig. 6. $a-v_{fem}$ difference of glucose (*A*) and lactate (*B*) and leg blood flow (*C*) from the study by Hermansen & Vaage (1977) (open symbols) and from the present study (filled symbols).

glucose uptake by the quadriceps muscle during the recovery. This was a result of smaller $a-v_{fem}$ differences and a markedly lower recovery leg blood flow (Fig. 6). The lower $a-v_{fem}$ lactate difference was likely due to a higher arterial lactate concentration, which has been shown to have an effect on lactate release and the

glycogen resynthesis from lactate (Stevenson, Mitchell, Hendrick, Rainey, Cherrington & Frizzell, 1987; Juel, Bangsbo, Graham & Saltin, 1990). The most critical factor is that they obtained blood flows with plethysmography of the calf after bicycle exercise. Whether these blood flows can be taken as representative for recovery limb blood flow after treadmill exercise which was used in the original study is open for question. It is most likely that they underestimated the flow and hence lactate release and glucose uptake. Subsequently the role of muscle lactate for glyconeogenesis was overestimated.

It is of note that the part of the lactate that remained in the muscle in the present study and was not oxidized could have supported a mean glycogen synthesis rate of 0·17 mmol glucosyl units $min^{-1}$ $kg^{-1}$ for the first 10 min of recovery. This rate is similar to the mean rate of glycogen synthesis from lactate of 0·19 mmol glucosyl units $min^{-1}$ $kg^{-1}$ that occurred in fast-twitch red and fast-twitch white muscle during perfusion of the rat hindlimb (Mclane & Holloszy, 1979). Whether the latter rate is a maximal value is unlikely as the dilution of the $^{14}C$ label within the gluconeogenic pathway may have caused underestimation amounting to a factor of two (Bronsman, 1982; Shiota *et al.* 1984; Johnson & Bagby, 1988).

It is also unlikely that maximal glycogen synthesis rates were reached in the present study. An inverse relationship exists between the glycogen concentration and glycogen synthesis in skeletal muscles (Piehl, Adolfsen & Nazar, 1974), and in the present study the glycogen depletion with one short bout of exercise was quite moderate. Furthermore, the rates measured in this study are the average for all muscle fibres, and it is known that glyconeogenesis is faster in fast-twitch than slow-twitch fibres (McLane & Holloszy, 1979; Bonen *et al.* 1990). The high muscle lactate and $CO_2$ concentrations at the end of exercise favour a large glyconeogenesis rate, but a fast decrease in these variables during early recovery (femoral venous $P_{CO_2}$: 95, 85 and 48 mmHg at 0, $\frac{1}{2}$ and 3 min of recovery, respectively) lowers the rate, and so the mean value for the first 10 min of recovery (Bendall & Taylor, 1970; Bonen *et al.* 1990). The reports of the effect of low pH on the synthesis of glycogen from lactate are conflicting. Bendall & Taylor (1970) found a reduced glycogen formation when pH was lowered in the bathing medium of isolated frog muscles, whereas Bonen *et al.* (1990) recently demonstrated that acidic conditions enhanced glycogen synthesis from lactate. Thus, the return of pH to pre-exercise level during early recovery was not optimal for converting lactate to glycogen during the entire first 10 min of recovery (Juel *et al.* 1990).

It is well-known that the $\dot{V}_{O_2}$ remains elevated after exercise, but the causes for this are unclear (Margaria, Edwards & Dill, 1933; Ceretelli, 1984; Gaessar & Brooks, 1984; Bahr, Ignes, Vaage, Sejersted & Newsholme, 1987). In the present study the $O_2$ debt for the whole body was 4·5 l of which the leg accounted for one-third or 1·5 l in the 60 min recovery period. Only a minor fraction (26 %) of the leg $O_2$ debt could be attributed to the resynthesis of ATP, CP and glycogen (Table 4). Increased catecholamine concentrations post-exercise and elevated temperature have been suggested to cause elevation in the post-exercise metabolic rate (Mæhlum, Grandmontagne, Newsholme & Sejersted, 1986). However, they are unlikely explanations in the present study as the changes in these variables are small during knee-extensor exercise. Moreover, both variables returned to control level within

20 min of recovery (Bangsbo, Gollnick, Graham, Juel, Kiens, Mizuno & Saltin, 1990). In spite of this, during the remaining 40 min the $\dot{V}_{O_2}$ of both the leg and whole body were still elevated.

Futile cycles may be a possible explanation for the higher $\dot{V}_{O_2}$ after exercise (Challiss, Arch & Newsholme, 1984; Mæhlum *et al.* 1986; Bahr, Hansson & Sejersted,

TABLE 4. Estimated energy demand of muscle resynthesis of ATP, CP and glycogen for the limb expressed in $O_2$ units (1 mmol ATP $\approx$ 4·5 ml $O_2$) and measured $\dot{V}_{O_2}$ and $O_2$ debt* during 1 h of recovery after intense exercise

| | Energy demand (ml $O_2$) | | | | | |
|---|---|---|---|---|---|---|
| | | Glycogen synthesis from | | | | |
| | ATP, CP resynthesis | Lactate† | Glucose + glycolytic intermediates | Total | Measured (ml $O_2$) $\dot{V}_{O_2}$ | Measured (ml $O_2$) $O_2$ debt |
| 0–10 min | 157 | 113 | 28 | 298 | 796 | 631 |
| 10–60 min | 43 | 8 | 70 | 121 | 1705 | 885 |
| 0–60 min | 200 | 121 | 98 | 419 | 2501 | 1516 |

Reloading of Hb and Mb $\approx$ 3·1 ATP $\approx$ 14 ml (0–10 min).
* $O_2$ debt = measured $\dot{V}_{O_2}$ – pre-exercise $\dot{V}_{O_2}$.
† Energy cost, 5 mmol ATP per 2 mmol lactate to glycogen (Bendall & Taylor, 1970).

1990). The glyconeogenic–glycolytical substrate cycle and the triacylglycerol–fatty acid substrate cycle have been shown to be present *in vivo* in man (Elia, Zed, Neale & Livesey, 1987; Wolfe, Herndon, Jahoor, Miyoshi & Woolfe, 1987; Wolfe & Peters, 1987; Miyoshi, Shulman & Peters, Wolfe, Elahi & Wolfe, 1988). These cycles are energy demanding, e.g. the net cost of one turn of the triacylglycerol–fatty acid substrate cycle is eight molecules of ATP (Brooks, Arch & Newsholme, 1983).

From the difference between the leg $O_2$ debt and estimated energy demand the extra energy consumption could be calculated to 7·4 and 3·4 mmol ATP $min^{-1}$ for the first 10 and last 50 min of recovery, respectively (Table 4). The metabolism of lactate, ADP, inorganic phosphate and creatine could account for only 47% of the $O_2$ debt from 0–10 min. The cause(s) of the rest of these portions of the debt are unknown, but could include reloading of Hb and Mb (about 3 mmol ATP $\approx$ 2%), energy for the gluconeogenic-glycolytical substrate cycle and such processes as the restoration of ionic homeostasis. During the rest of recovery the extra energy consumption may be linked with fat turnover. The leg RQ was below 0·70 and RER was 0·71 after 10 min of recovery, which indicates pure fat oxidation in the muscle. At the same time there was no net uptake of FFA over the thigh. This does not exclude FFA being taken up by the muscle but an equivalent amount must then have been released. Further with fatty acids continuously being utilized by the mitochondria, triacylglycerol within the muscle must have been the source of the fatty acids. This is supported by a net glycerol release (Table 3B). Thus, the triacylglycerol–fatty acid substrate cycle might account for a significant portion of the observed elevated $\dot{V}_{O_2}$ for the last 50 min.

In summary, more than two-thirds of the lactate that accumulated in the muscle during the intense exercise was released to the blood, and a minimum of 13% and

a maximum of 27% was converted to glycogen. Glucose taken up from the blood stream and glycolytic intermediates accumulated during the exercise were the dominant substrates for glycogen synthesis. Furthermore, intramuscular triacylglycerol appeared to be the major source of the fat oxidation during recovery, and the triacylglycerol–fatty acid substrate cycle is suggested to account for a significant part of the $O_2$ debt of the leg in recovery after exercise.

The study was made possible by a grant from the Danish Natural Science Research Council, grant no. 11–7766, Team Danmark and Brandt legat. T. Graham was supported by NSERC (Canada) international collaborative research grant.

## REFERENCES

ANDERSEN, P., ADAMS, R. P., SJØGAARD, G., THORBOE, A. & SALTIN, B. (1985). Dynamic knee extension as a model for the study of an isolated exercising muscle in man. *Journal of Applied Physiology* **59**, 1647–1653.

ANDERSEN, P. & SALTIN, B. (1985). Maximal perfusion of skeletal muscle in man. *Journal of Physiology* **366**, 233–249.

ÅSTRAND, P.-O., HULTMAN, E., JUHLIN-DANFELT, A. & REYNOLDS, G. (1986). Disposal of lactate during and after strenuous exercise. *Journal of Applied Physiology* **61**, 338–343.

BAHR, R., HANSSON, P. & SEJERSTED, O. M. (1990). Triglyceride/fatty acid cycling is increased after exercise. *Metabolism* **39**, 993–999.

BAHR, R., INGNES, I., VAAGE, O., SEJERSTED, O. M. & NEWSHOLME, E. A. (1987). Effect of duration of exercise on excess postexercise $O_2$ consumption. *Journal of Applied Physiology* **62**, 485–490.

BANGSBO, J., GOLLNICK, P. D., GRAHAM, T. E., JUEL, C., KIENS, B., MIZUNO, M. & SALTIN, B. (1990). Anaerobic energy production and $O_2$ deficit–debt relationship during exhaustive exercise in humans. *Journal of Physiology* **422**, 539–559.

BENDALL, J. R. & TAYLOR, A. A. (1970). The Meyerhof quotient and the synthesis of glycogen from lactate in frog and rabbit muscle. *Biochemical Journal* **118**, 887–893.

BONEN, A., MCDERMOTT, J. C. & TAN, M. H. (1990). Glycogenesis and glyconeogenesis in skeletal muscle: effects of pH and hormones. *American Journal of Physiology* **21**, E693–700.

BRONSMAN, J. T. (1982). Pathways of carbon flux in gluconeogenesis. *Federation Proceedings* **41**, 91–95.

BROOKS, G. A. (1985). Anaerobic threshold: review of the concept and directions for future research. *Medicine and Science in Sports and Exercise* **17** (1), 22–31.

BROOKS, B. J., ARCH, J. R. S. & NEWSHOLME, E. A. (1983). Effect of some hormones on the rate of triacylglycerol/fatty acid substrate cycle in adipose tissue of the mouse *in vivo*. *Bioscience Reports* **3**, 263–267.

CERETELLI, P. (1984). Oxygen debt; definition, role and significance. *Medicine and Sport Science* **17**, 68–80.

CHALLISS, R. A. J., ARCH, J. R. S. & NEWSHOLME, E. A. (1984). The rate of substrate cycling between fructose 6-phosphate and fructose 1,6-biphosphate in skeletal muscle. *Biochemical Journal* **221**, 153–161.

CHEETHAM, M. E., BOOBIS, L. H., BROOKS, S. & WILLIAMS, C. (1986). Human muscle metabolism during sprint running. *Journal of Applied Physiology* **61**, 54–60.

DOUGLAS, A. R., JONES, N. L. & REED, J. W. (1988). Calculation of whole blood $CO_2$ content. *Journal of Applied Physiology* **65** (1), 473–477.

DRABKIN, D. L. & AUSTIN, J. H. (1935). Spectrophotometric studies II. Preparations from washed blood cells, nitric oxide hemoglobin and sulfhemoglobin. *Journal of Biological Chemistry* **122**, 51–65.

ELIA, M., ZED, C., NEALE, G. & LIVERSEY, G. (1987). The energy cost of triglyceride–fatty acid recycling in nonobese subjects after an overnight fast and four days of starvation. *Metabolism* **36**, 251–255.

FELIG, P. & WAHREN, J. (1974). Amino acids metabolism in exercising man. *Journal of Clinical Investigation* **50**, 2703–2714.

GAESSER, G. A. & BROOKS, G. A. (1984). Metabolic bases of excess postexercise oxygen consumption: a review. *Medicine and Science in Sports Exercise* **16**, 29–43.

GAFFNEY, F. A., SJØGAARD, G. & SALTIN, B. (1990). Cardiovascular and metabolic responses to static contraction in man. *Acta Physiologica Scandinavica* **138**, 249–258.

HARRIS, R. C., HULTMAN, E. & NORDESJÖ, L. (1974). Glycogen glycolytic intermediates and high-energy phosphates determined in biopsy samples of musculus quadriceps femoris of man at rest. *Scandinavian Journal of Clinical Laboratory Investigation* **33**, 109–120.

HEINRIKSON, R. L. & MEREDITH, S. C. (1984). Amino acid analysis by reverse-phase high-performance liquid chromatography: Precolumn derivatization with phenylisothiocyanate. *Analytical Biochemistry* **136**, 65–74.

HERMANSEN, L. & VAAGE, O. (1977). Lactate disappearance and glycogen synthesis in human muscle after maximal exercise. *American Journal of Physiology* **233**, E422–429.

HOLMGREN, A. & PERNOW, B. (1959). Spectrophotometric determination of oxygen saturation of blood in the determination of cardiac output. *Scandinavian Journal of Clinical Laboratory Investigation* **11**, 143–149.

JOHNSON, J. L. & BAGBY, G. J. (1988). Gluconeogenic pathway in liver and muscle glycogen synthesis after exercise. *Journal of Applied Physiology* **64**, 1591–1599.

JONES, N. L., MCCARTNEY, N., GRAHAM, T., SPRIET, L. L., KOWALCHUK, J. M., HEIGENHAUSER, J. F. & SUTTON, J. R. (1985). Muscle performance and metabolism in maximal isokinetic cycling at slow and fast speeds. *Journal of Applied Physiology* **59**, 132–136.

JONES, P. R. M. & PEARSON, J. (1969). Anthropometric determination of leg fat and muscle plus bone volumes in young male and female adults. *Journal of Physiology* **204**, 63*P*.

JUEL, C., BANGSBO, J., GRAHAM, T. & SALTIN, B. (1990). Lactate and potassium fluxes from human skeletal muscle during and after intense, dynamic knee extensor exercise. *Acta Physiologica Scandinavica* **140**, 147–159.

KREBS, H. A. & WOODFORD, M. (1965). Fructose 1,6-diphosphate in striated muscle. *Biochemical Journal* **94**, 436–445.

LOWRY, O. H. & PASSONNEAU, J. V. (1972). *A Flexible System on Enzymatic Analysis*. Academic Press, New York.

MCCARTNEY, N., SPRIET, L. L., HEIGENHAUSER, J. F., KOWALCHUK, J. M., SUTTON, J. R. & JONES, N. L. (1986). Muscle power and metabolism in maximal intermittent exercise. *Journal of Applied Physiology* **60** (4), 1164–1169.

MCLANE, J. A. & HOLLOSZY, J. O. (1979). Glycogen synthesis from lactate in three types of skeletal muscles. *Journal of Biological Chemistry* **254**, 6548–6553.

MÆHLUM, S., GRANDMONTAGNE, M., NEWSHOLME, E. A. & SEJERSTED, O. (1986). Magnitude and duration of excess post-exercise oxygen consumption in healthy young subjects. *Metabolism* **35**, 425–429.

MAGNSON, N. S. & PERRYMAN, L. (1980). Adenine nucleotides in erythrocytes of horses. *Equus cabalus. Comparative Biochemistry and Physiology* **67B**, 205–221.

MARGARIA, R., EDWARDS, H. T. & DILL, D. B. (1933). The possible mechanism of contracting and paying the oxygen debt and the role of lactic acid in muscular contraction. *American Journal of Physiology* **106**, 689–714.

MEYERHOF, O. (1920). Uber die Energiumwandlungen in Muskel, II. Das Schicksal der Erholungsperiode des Muskels. *Pflügers Archiv* **182**, 284–317.

MIYOSHI, H., SHULMAN, G. I., PETERS, E. J., WOLFE, M. H., ELAHI, D. & WOLFE, R. R. (1988). Hormonal control of substrate cycling in humans. *Journal of Clinical Investigation* **81**, 1545–1555.

OPIE, L. H. & NEWSHOLME, E. A. (1967). The activities of fructose 1,6-diphosphatase, phosphofructokinase, and phosphoenolpyruvate carboxykinase in white muscle and red muscle. *Biochemical Journal* **103**, 391–399.

PIEHL, K., ADOLFSEN, S. & NAZAR, K. (1974). Glycogen storage and glycogen synthetase activity in trained and untrained muscle of man. *Acta Physiologica Scandinavica* **90** 779–788.

RICHTER, E. A., MIKINES, K. J., GALBO, H. & KIENS, B. (1989). Effect of exercise on insulin action in human skeletal muscle. *Journal of Applied Physiology* **66**, 876–885.

SALTIN, B. (1985). Hemodynamic adaptations to exercise. *American Journal of Cardiology* **55**, 42–47D.

SHIMIZU, S., INOUE, K., TANI, Y. & YAMADA, H. (1979). Enzymatic microdetermination of serum free fatty acids. *Analytical Biochemistry* **98**, 341–345.

SHIOTA, M., GOLDEN, S. & KATZ, J. (1984). Lactate metabolism in the perfused rat hindlimb. *Biochemical Journal* **222**, 281–292.

SIEGEL, S. (1965). *Nonparametric Statistics for the Behavioral Sciences*. McGraw-Hill, New York.

STEVENSON, R. W., MITCHELL, D. R., HENDRICK, G. K., RAINEY, R., CHERRINGTON, A. D. & FRIZZELL, R. T. (1987). Lactate as substrate for glycogen resynthesis after exercise. *Journal of Applied Physiology* **62** (6), 2237–2240.

WESCHE, J. (1986). The time course and magnitude of blood flow changes in the human quadriceps muscles following isometric contraction. *Journal of Physiology* **377**, 445–462.

WOLFE, R. R., HERNDON, D. N., JAHOOR, F., MIYOSHI, H. & WOLFE, M. (1987). Effect of severe burn injury on substrate cycling by glucose and fatty acids. *New England Journal of Medicine* **317**, 403–408.

WOLFE, R. R. & PETERS, E. J. (1987). Lipolytic response to glucose infusion in human subjects. *American Journal of Physiology* **252**, E218–223.